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Effects of mixing on the result of anaerobic digestion: Review

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ABSTRACT

Mixing in an anaerobic digester keeps the solids in suspension and homogenizes the incoming feed with the active microbial community of the digester content. Experimental investigations have shown that the mixing mode and mixing intensity have direct effects on the biogas yield even though there are conflicting views on mixing design. This review analyzes and presents different methods to evaluate the mixing in a digester (chemical and radioactive tracers and laboratory analysis), tools for digester design (computational fluid dynamics and kinetic modeling) and current research on the effects of mixing on the anaerobic digestion process. Empirical data on experiments comparing different mixing regimes have been reviewed from both a technical and microbial standpoint with a focus both on full scale digesters and in lab-scale evaluations. Lower mixing intensity or uneven mixing in the anaerobic digestion process can be beneficial during the startup phase to allow for methanogenic biomass growth and alleviate process instability problems. Intermittent mixing has been shown to be able to yield a similar gas production as continuous mixing but with the possibility to reduce the maintenance and energy demands of the process. Problems often experienced with experimental design include the effect of mixing on the solids retention time, and measurement of steady state gas production because of startup instabilities. Further research should be aimed at studying the effects of mixing on a chemical and microbial level and on the different stages of anaerobic digestion (hydrolysis, acidogenesis, acetogenesis and methanogenesis). The focus should be on the effects of mixing on a multiple stage digestion process and also finding new methods to evaluate the effects of mixing in the one stage digestion process rather than evaluating a wider range of mixing modes, intensities and substrates.

Contents

Abbreviations: AD, anaerobic digestion; CARPET, computer automated radioactive particle tracking; CFD, computational fluid dynamics; COD, chemical oxygen demand; HRT, hydraulic retention time; OLR, organic loading rate; SRT, solid retention time; TS, total solids; VFA, volatile fatty acids

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1. Introduction

The anaerobic digestion (AD) process has been studied intensively over the last few decades for its application on biomass and solid waste digestion and for its use in industries such as waste water treatment and agriculture [26,57,94,3,58,16]. Understanding and control of the digester and the biological processes within it are of great importance in order to improve the AD process and increase biogas production in industrial biogas plants.

Considerable effort has focused on selection of the right substrate or mixture of substrates, pretreatment to remove contaminants, reducing substrate size and hygienization in the AD industry, but very little effort has been put into controlling the digestion itself. The continuously stirred tank reactor (CSTR) is a very common digester design where the content is mixed continuously to maintain the solids in suspension and to form a homogenous mixture. Mixing mode and intensity are important control measures for the CSTR and many investigations have shown that they have direct effects on the biogas yield, even though there are conflicting views on how the mixing should be designed. Positive effects on the biogas yield have been achieved both by increasing [35] and decreasing [80,89] mixing in the AD process.

The AD process is dependent on mixing for distribution of microorganisms and nutrition, inoculation of fresh feed, homogenization of the material, removal of end products of metabolism and for evening out the temperature inside the digester [22]. Microorganisms are sensitive to mixing intensity and may not survive excessively forceful mixing [22]. In this review we analyze and evaluate the results of a number of studies with the aim of formulating general rules and empirical relations between mixing and gas production in the AD process.

2. Modes of mixing

There are a number of different types of mixing equipments used in the AD industry. These include mechanical mixing, hydraulic mixing and pneumatic mixing [22]. Mechanical mixing is the most common mixing type being used in Europe today, and uses different types of propellers and agitators to homogenize the digester content. Mechanical mixing has also been reported as having the highest power efficiency per volume unit mixed [9]. Hydraulic mixing is performed via pumps located outside the digester, which recirculate the AD sludge, and pneumatic mixing utilizes the gas, which is pumped back down into the digester and released to create a horizontal mixing action as the bubble column rises to the surface. The quality and results of mixing can vary depending on the equipment used and the geometry of the digester.

Aside from the mixing equipment used, mixing intensity and alternative mixing modes can be used to further influence the process. The mixing intensity can be defined as the power used per unit volume, but is often presented as rotational speed of a mixer, or as the flow output of a pump (gas or liquid). Regardless of how the mixing intensity is presented, a lower mixing intensity can save energy and improve the energy balance of the system. The United States Environmental Protection Agency (EPA) gave a general recommendation of 5–8 W/m^3 of digester volume in 1979 (US EPA), but there is no real consensus on how much mixing is actually needed.

The digester can either be mixed continuously, using an intermittent mixing mode, or not be mixed at all. Intermittent mixing means that mixing is turned on and off according to a preset time interval that can range from a few seconds of mixing per day to an almost continuous mixing mode. The power demand of the mixer motor increases during startup of mixing, and this increase needs to be considered when considering energy optimization of the process. Kowalczyk et al. [43] reported a 2.5% average increase in power consumption by the mixer motor for the first 13 min following startup in their laboratory-scale setup. The energy demand for mixing in a full-scale digester is substantial and can vary from 14% to 54% of the total energy demand of the plant [70,43]. Kowalczyk et al. [44] reported that the energy demand of mixing could be reduced by 12–29% if intermittent mixing was used instead of continuous mixing. By applying an intermittent regime, mixing for 2 h and pausing mixing for 1 h, the energy demand was reduced by 29% compared to a continuous mixing mode, and when mixing for 7 h and pausing mixing for 1 h, the energy demand was reduced by 12%.

Gas release from the liquid digestate in intermittently mixed digesters has been shown to increase by up to 70% during mixing periods [82,65,61]. This implies that gas release is impeded in the unmixed condition and that mixing increases the mass transfer of the gas from the liquid phase to the gas phase. Stafford [79] analyzed the gas transfer from liquid to gas phase during different mixing regimes (140–1000 rpm intermittent mixing) and demonstrated gradually increasing gas release with increasing mixing intensity during the first minute of mixing.

Depending on the type of substrate treated in the AD process the feed can have different properties. Light materials such as feathers and straw have a tendency to float on the surface [15], while heavier materials like egg shell and other heavy particles sink to the bottom. Stratification processes such as sedimentation and floatation are often seen as a problem in the digestion process, which can lead to reduced active digester volume, mechanical problems with pumps and stirrers, and obstruction of the release of biogas from the liquid phase. Keeping the digester content in suspension by mixing can solve many of these problems as well as evening out the feed concentration and temperature gradient in the digester. Foaming on the surface is also a problem that can occur in the absence of mixing [80] or during mixing breaks [43], but no negative effect on the gas yield has been reported. Indeed, Stroot et al. [80] describe an increase in active volume. The use of a hopper bottom digester and local agitation of the surface to reduce sedimentation, flotation and foaming is a possible solution, but an evaluation of each individual substrate is needed to identify problems that may occur. It is important to remember that these

operational problems also occur in a CSTR and are not exclusive to the unmixed and intermittently mixed digester.

2.1. Mixing and the microbial community

High mixing intensity and the resultant shear stress has a negative effect on flock formation and can inhibit gas production [93,59,80,41]. Close proximity between some microorganisms in flocs, often referred to as juxtapositioning, is important for syntrophic interaction. Interspecies hydrogen transfer is one important syntrophic interaction, where hydrogen is transferred directly between acetogenic and methanogenic (producers and consumers of hydrogen) microorganisms to keep the partial hydrogen pressure low, which would otherwise affect the AD negatively [20,21]. Intensive mixing has been shown to break up flocks and reduce extracellular polymeric substances (EPS), which aid adhesion between cells and between cells and other surfaces [65]. Hoffman et al. [32] observed the destruction of almost all flocs during continuous stirring at a wide range of mixing intensities (50–1500 rotations per minute, rpm) without any negative effects on biogas production. Floc destruction is attributed to shear stress created by mixing. Excessive mixing intensity can increase the startup time of the biogas process, due to process instabilities with high propionate and acetate values [37], conversely reducing the mixing intensity can stabilize an unstable process [33].

Mixing also has an effect on the consortia of the microbial community. McMahon et al. [59] and Hoffman et al. [32] reported an increase in *Methanosarcina* spp. and Methanobacteriacea during a destabilization of the AD process caused by a higher mixing intensity. *Methanosarcina* spp. increased in digesters with periodically high acetate levels and were abundant in all digesters with a history of high volatile fatty acid (VFA) values, while *Methanosaeta concilii* only increased in abundance in digesters mixed at lower intensities where there was no VFA accumulation. According to Hoffman et al. [32] the reason for the inverse correlation between these species is that they compete for acetate, but *Methanosarcina* are more of a generalist and thrives where there is higher acetate concentration whereas *M. concilli* are more specialized and requires lower acetate levels. Mixing can also have a direct effect on the microbial community due to differences in cell morphology, since *M. concilli* form long filaments and Methanosarcina spp. are cocci. The filaments of *M. concilli* may be more vulnerable to mechanical damage from the mixing [32]. McMahon et al. [59] also observed that *Syntrophobacter wolinii* are responsible for propionate degradation during digester stabilization and that they are abundant in digesters that have a history of high VFA values in continuously mixed conditions.

Methanogenic microorganisms have a much longer regeneration time (10–15 days) compared to hydrolytic (24–36 h) and acid-forming microorganisms (80–90 h) in the biogas process, making them more susceptible and sensitive to washout and inhibition [22]. According to Vavilin and Angelidaki [89], uneven mixing can create initiation zones where methanogens can grow and thrive and from where they can seed the rest of the digester. Spatial separation between acidogenic and methanogenic zones is necessary when methanogenesis is the rate limiting step to protect methanogens from over-acidification during high organic loading rate (OLR) or during startup of the process. However, if the hydrolysis becomes rate limiting, high mixing can increase biogas production and substrate degradation. Vavilin et al. [90] showed that high intensity mixing inhibits methanogenesis and hydrolysis/acidogenesis and that the result of AD is dependent on the methanogenic biomass concentration. A gradual increase in OLR can allow the methanogenic biomass to grow and improve the startup of a completely mixed reactor. After the initial startup phase or lag phase, an increase in mixing aids the mass transfer of nutrient and accelerates solid waste digestion [88]. The separation of hydrolysis and acidogenesis from acetogenesis and methanogenesis to prevent this type of acidification problems is taken to its extreme in the two-stage AD system where physical walls protect the methanogens. Stroot et al. [80] also hypothesized that the syntrophs and methanogens could have difficulties degrading the fermentation products at higher OLR and that by reducing the mixing the hydrolysis and fermentation would slow down to allow the products to be consumed without accumulation of acids and their inhibiting effects.

3. Evaluating the mixing

Many studies have been performed to evaluate different types of mixing equipment and digester designs used in the AD process and other types of bioreactors. This includes studies of tracer methods, different laboratory experiments as well as modeling. The understanding of the fluid flows in the reactor needs to be improved in order to generate fundamental knowledge about the influence of mixing on biogas production. With better knowledge of the relationship between mixing and biogas production, optimizations of the process performance should be possible.

3.1. Tracer methods

Two different types of tracer methods for the evaluation of the mixing quality are presented here. The chemical tracer method is based on the concentration response curve of an injected tracer in the reactor effluent. Radioactive particle tracking is based on sensor technology which can pick up the gamma ray photons produced by the injected particles.

3.1.1. Chemical tracers

Evaluating the mixing in an operational digester can be performed by adding a known mass of a tracer (e.g. lithium or fluoride) and studying the concentration change of the tracer in the effluent stream using an ion selective electrode, spectroscopy or other chemical analysis. The tracer can be added as a pulse, instantaneously injected and monitored until the tracer concentration has reached zero, or as a step with continuous addition of the tracer until the concentration in the effluent has stabilized [14]. The data collected can be used to determine the residence time distribution (RTD). This type of evaluation can provide information about stagnant regions, shortcircuiting, breakthrough time and RTD. The shape of the RTD curve reveals any problems with the mixing. A perfectly mixed CSTR would result in a smooth exponential decay with the observed mean tracer concentration close to the hydraulic retention time (HRT) following a pulse injection of tracer chemical. However, if the mean tracer concentration in the effluent is observed before the theoretical HRT of the reactor, this may indicate stagnant regions. Alternatively, a sharp early peak in the tracer concentration may indicate shortcircuiting problems [47]. A suitable mixing model can be implemented to extract more exact information from the RTD curve. Monteith and Stephenson [62] described a model based on the work of Cholette and Cloutier [19] and Levenspiel [46] for a CSTR (1) with, and (2) without short-circuiting, including a mixed region and a stagnant region which is unavailable for mixing:

$$C/C_0 = [(v_1 V/v_2)/\Sigma t_a] \exp\{-t/\Sigma t_a\} + (v_2/v)\delta_{t=0} \quad (1)$$

$$C/C_0 = (V/V_a)\exp\{-t[V/(V_a\tau)]\} \quad (2)$$

where C and C_0 are the tracer concentration at time t and the expected concentration at the start respectively, V is the digester volume, v is the total flowrate, v_1 is the flowrate of the mixed zone, v_2 is the shortcircuting flowrate, t is the time in days, Σt_a is the mixed zone mean HRT and δ is the Dirac delta function. Monteith and Stephenson [62] implemented this model in their work and reported on the

mixing efficiency in two full-scale two-stage anaerobic digesters using sodium fluoride as a tracer. The result of the study exposed major shortcomings in the mixing including large unmixed zones, low HRT and shortcutting flows. They also presented conclusions from similar studies performed by Tenney and Budzin [84] and Smart [75] showing large inconsistencies in the mixing. Capela et al. [14] studied the RTD in a full scale reactor treating evaporator condensate effluent from a sulfite pulp mill and reported an unmixed region taking up 22% of the reactor volume which contributed to the presence of large amounts of precipitates.

Levenspiel [47] also presented the dispersion model and the tank-in series model which are models for near plug flow circumstances that can also be modified for use in other circumstances. The models presented by Levenspiel have been implemented and developed by a number of researchers [12,56,14]. The tracer method is very versatile and can be used for a wide range of different industrial applications and treatments including e.g. the activated sludge process [12], upflow anaerobic filters [78], fixed film anaerobic reactors [23], membrane bioreactors [10] and anaerobic contact processes [77]. Setting up and executing this type of evaluation using a tracer is very time consuming and the evaluation of the results requires some experience to be able to extract all the information from the RTD curve.

3.1.2. Radioactive particles

Computer automated radioactive particle tracking (CARPET) is a method that is used to study the movement of a radioactive particle inside a laboratory digester. By tracking the movement of the particle, the mixing of the digester can be evaluated in terms of hydrodynamics, particle flow patterns, liquid velocities, shear stress and stagnant zones. This technique has been used successfully by Karim et al. [34], Vesvikar and Al-Dahhan [91], Hoffmann et al. [32] and their group at Washington University in St. Louis to study mixing in lab-scale digesters and as verification for their further studies using CFD. In this method the radioactive isotope Sc-46 particle is coated with paralyne, activated and sealed in a plastic or metal sphere to give it similar diameter, density and hydrodynamic properties as the solid particles being studied but with the crucial difference that it emits gamma ray photons [72,32]. The movement of the particle can then be tracked by an array of NaI scintillation detectors. Zoltek and Gram [99] studied the mixing in full scale reactors for waste water treatment using the radioactive isotope Na-24 and two scintillation detector systems. Other studies of digester mixing using radioactive particles were performed by Loffell [52] and White [92].

3.2. Laboratory analysis of mixing

Different types of laboratory experiments can be performed to study the effects of mixing as there is the possibility to use transparent glass or acrylic digesters and for adding sensor equipment of different types. One straightforward approach is to use a dye, acid–base neutralization reaction or added particles to allow visual observation of the effects of the mixing equipment, mixing intensity and mode of mixing on the flow fields. The results can also be evaluated by using a camera and different techniques for image analysis. This type of analysis requires transparent digester walls and a clear liquid phase with a viscosity close to the studied medium for best results. Since digestion sludge is usually opaque, water or a synthetic liquid is often used to represent the digester content. Low et al. [53] performed a hydrodynamic study of a digester using a synthetic model fluid made up from Xanthan gum Keltrol T powder mixed in water in different concentrations and a video camera.

The chemical engineering field uses a wide range of different flow-visualization techniques to study mixing and to evaluate isolated mixing regions [54,45,29], different types of stirrers/impellers [64], different speed modulations [98], movements of particles inside a digester [1], and mixing time [13]. Other methods include the use of different probes in the tank but this can affect the flow field of the reactor since the probe itself creates an obstacle which diverts the flow from its normal path. However probes are still a useful tool to study mixing and they include the use of pitot tube probes [27], hot-film probes [27] and optical probes [17,42,27], among many others. The pitot tube measures the dynamics between the static and total pressures, the hot-film probe measures the heat transfer rate from the probe to the surroundings and optical probes are usually used in multiphase flows to study the size and speed of bubbles by detecting changes in refraction and reflection. The use of different sensors, probes and visual techniques is an entire research field in its own right and guidance on the selection of probes for different applications can be found in scientific publications in that field. There are very few opportunities to perform these kinds of measurements in full scale digesters since the process is oxygen free and there are usually few access ports into the digester. However, these types of experiments are valuable when designing the mixing in a new AD system but scale up factors have to be considered.

4. Effects of mixing

The effects of mixing have been evaluated in a number of studies. These studies include important control parameters such as the OLR and the effect of mixing on the retention time and on the selected mixing mode (see Section 2). General guidelines for mixing can be formulated by comparing the results from different mixing experiments.

4.1. Control parameters

Research on mixing efficiency has focused on two main parameters, namely the retention time and organic loading.

4.1.1. Retention time

The HRT is a measure of the average retention time of a liquid or dissolved component inside a reactor and it is calculated as the tank volume divided by the influent flow rate. In the AD process the HRT is used to approximate the time during which the substrate is anaerobically treated. The HRT is often used synonymously with the solid retention time (SRT) in the AD industry, which in practice means that the reactor content should be mixed perfectly to distribute the feed/substrate to produce a homogeneous slurry within the reactor, which is rarely the case. The actual HRT and the SRT are both controlled by the mixing and depending on how the digester is mixed they may have a large effect on the gas yield. This does not mean that a well-mixed digester is always preferred, but it is important to understand the effect of the implemented mixing for control purposes. In an experiment performed by Ong et al. [65] it was concluded that the stratification of the substrate in an unmixed digester decoupled the SRT from the HRT and increased the SRT of the material and the biogas production compared to a perfectly mixed reactor at the same HRT, since fewer particles were removed with the effluent. The HRT can vary greatly between different processes and the type of substrate used, from a couple of days to a couple of months.

4.1.2. Organic loading rate

The OLR is a measure of the amount of substrate/feed that is added to a continuous digester system per unit of volume and day.

The OLR is often presented as grams of volatile solids (VS), total solids (TS) or chemical oxygen demand (COD) per liter digester volume and day. If the OLR is too high or if a sudden shock load of feed is added to a system it will destabilize, resulting in an increase in VFA and a decrease in gas production. Karim et al. [37] concluded that mixing was an important aspect at higher TS content (read as OLR) but had no apparent effect at lower concentrations. The amount of substrate added and the type of substrate used also changes the rheological property and the viscosity of the digestate. Increased viscosity means that a higher mixing intensity is needed to achieve the same result.

4.2. Empirical results

A wide range of experiments has been performed to study the effect of mixing on the production of biogas and methane as well as the reduction of TS, VS and COD. These experiments have used different setups including lab-scale, pilot-scale and batch assays with different modes of mixing. The mixing has been performed continuously or intermittently using gas mixing, slurry recirculation, shaker tables and different impellers and stirrers at a wide range of flows and speeds. Experimental data from a number of these studies is presented in Figs. A1 and A2 and Tables A1 and A2 in Appendix A and summarized here in Table 1, which excludes articles with unclear outcomes. Most of these experiments were performed at lab-scale and in the mesophilic temperature interval, and most attention thus far has been given to the digestion of cattle manure. Table 1 shows varying results from comparing continuous mixing to unmixed digesters. However, the results from the different studies show that an intermittent mixing regime enhances the AD compared to a continuously mixed system.

When comparing different intermittently mixed systems, there appears to be no disadvantage to keeping the mixing period short. Sulaiman et al. [81] noticed decreased biogas production when increasing mixing from 30 min per 6 h to 30 min per 2 h using sludge recirculation (125 m^3/h pump) and Lin and Pearce [48] showed an increase in biogas production when reducing mixing from 45 min/h to 15 min/h. Conversely, Kowalczyk et al. [43] did not observe differences dependent on the intermittent mixing regimes. Similar results were obtained from regimes with (1) mixing for 2 h and non-mixing for 1 h; or (2) mixing for 7 h and non-mixing for 1 hour. Similarly, gas production was the same with (1) mixing for 10 min per 4 hours; and (2) mixing for 10 min/h. Stafford [79] reported a small reduction in gas production when mixing at intensities above 700 rpm (700–1000 rpm) during intermittent mixing regimes while lower mixing intensities (140–425 rpm) all resulted in similar gas production when treating primary sewage sludge.

The effect of mixing intensity is not clear and seems to be substrate dependent. Hamdi [28] found different effects of mixing on raw substrate compared to pretreated substrate. However, as Table 1 shows, many researchers have found that changing the mixing intensity does not affect production. Hoffmann et al. [32] and Ong et al. [65] noticed no difference in gas production from varying mixing intensity from 50 to 1500 rpm (impeller) and 100–200 rpm (impeller) respectively, while Jiaja et al. [18] and Hamdi [28] found that the best results of mixing were obtained at 120 rpm (impeller) and 50–200 rpm (shaking table) respectively, which were the highest intensities used in these experiments.

The intense mixing during shock loads or during startup of the digestion process has been shown to have negative effects on biogas and methane production [37,32]. VFA concentration in the digestate increases during high intensity mixing [80,81]. This process instability also appears to occur at lower mixing intensities with higher OLR [32]. Lindmark et al. [51] also observed a decrease in biogas production during intense mixing. However, the effects could not be related to VFA inhibition since the production of VFA was reduced rather than increased. According to these results destabilization of the process, as shown by VFA accumulation, under intense mixing may not be the only reason why gas production is inhibited.

Rico et al. [69] and Gomez et al. [25] concluded that the greatest benefit of shifting mixing from continuous to an intermittent mixing mode was not the increase in gas production but that similar gas production could be achieved while using less energy, making the process more energy efficient. Hashimoto [30] concluded that the increased energy demand of continuous mixing compared to intermittent mixing was not justifiable in terms

Table 1
Comparison between the effects of different mixing.

	Feed	Continuous	Unmixed	Intermittent	Intensity (x)		Mixing period (y)	
					High	Low	Short	Long
Karim et al. [37]	A	1	0	–	–	–	–	–
[69]	A	0	–	1	–	–	–	–
Kaparaju et al. [33]	A	0	–	1	–	–	–	–
[33]	A	0x	–	1y	0	1	1	0
Stroot et al. [80]	B	0	–	1	–	–	–	–
Sulaiman et al. [81]	C	–	0	0y	–	–	1	0
[32]	A	*x*	–	–	0	0	–	–
Karim et al. [36]	A	1	0	–	–	–	–	–
Karim et al. [35]	A	0*x*	0	–	0	0	–	–
Ong et al. [65]	A	0*x*	–	0	0	0	–	–
Kim et al. [41]	D	0	1	–	–	–	–	–
Rojas et al. [71]	E	1	0	–	–	–	–	–
[48]	F	–	0	1*xy*	0	0	1	0
Hamdi [28]	G	0*x*	1		0	1	–	–
Hamdi [28]	H	1*x*	0	–	1	0	–	–
Chen et al. [18]	I	*x*	–	–	1	0	–	–
Kowalczyk et al. [43,44]	J	0	–	0*x*	–	–	0	0
[24]	K	0	–	0	–	–	–	–
Lindmark et al. [51]	K	1*x*	0	–	0	1	–	–
Keanoi et al. [40]	L	1	0	–	–	–	–	–

0 indicates the worst result, or the baseline, of the mixing experiment and 1 indicates an improvement over the baseline. Different mixing intensities (x) and mixing periods (y) were compared in some of these studies and are indicated by the letters x and y where they are applied. The different feeds used are: (A) cow manure, (B) primary sludge and waste activated sludge, (C) palm oil mill effluent, (D) dog food, (E) lipid-rich mix (kitchen, meat and milk-processing) and corn silage, (F) primary clarifier influent (potato processing), (G) olive mill wastewater, (H) fermented olive mill wastewater (fermented by fungi), (I) rice straw, (J) maize silage, corn cob mix and cow manure, (K) source-sorted organic fraction of municipal solid waste, and (L) cow dung, rice straw and water hyacinth mix.

of yield. Ong et al. [65] attained similar gas production from continuous and intermittent mixing and attributed any increase in gas production in their series of experiments to an increase in SRT caused by the mixing. By reducing mixing from continuous to intermittent, gas production can be increased [33] while modulating process instabilities [80,59]. Lin and Pearce [48] reported increased methane production from intermittent mixing compared to unmixed digesters while Hashimoto [30] reported contrasting experimental results in his lab-scale setup. Mixing experiments performed in two pilot scale/full scale digesters demonstrated similar gas production in continuously mixed (160 rpm) and intermittently mixed (160 rpm, 2 h/d) setups [30], and increased gas production by reducing the mixing period [81].

Karim et al. [36,37] compared different mixing equipment: gas mixing, slurry recirculation and impeller mixing to an unmixed setup, using the same power input of 8 W/m^3 as recommended by the EPA [87]. According to these results the different mixing equipment (and the unmixed setup) had similar effects during low OLR but mixing improved gas production at higher OLRs compared to the unmixed alternative. The need for mixing has been shown to be affected by the TS content of the feed and digestate since this affects the viscosity and rheological properties [35–37]. Passive mixing appears to be sufficient for AD processes running at lower TS contents but mixing becomes increasingly important with higher TS values [35]. Keanoi et al. [40] reached similar results and also observed an increased biogas production as a result of mixing during higher TS contents. Unmixed digesters have also shown increased biogas production compared to continuously mixed digesters in both mesophilic [28] and thermophilic [41] conditions. However, when Hamdi [28] used fungi for fermentation in the olive mill wastewater (OMW) digester, thereby altering its properties, gas production increased with mixing intensity. This was attributed to the decrease in toxic compounds in the substrate. Similarly, Karim et al. [36,37] digested cow manure, Kim et al. [41] digested dog food and Hamdi [28] digested OMW and found that digestion processes with different substrates (with different properties) responded differently to mixing.

Other types of digesters also exhibit similar effects from mixing. Sung and Dague [82] evaluated the use of different modes of mixing in the anaerobic sequencing batch reactor (ASBR) treating a synthetic substrate made up of nonfat dry milk, showing improved methane production and similar COD removal during intermittent mixing (5 min/h, 2.5 min/30 min and 100 s/20 min) compared to continuous mixing, using biogas recirculation for mixing.

5. Modeling

A wide variety of modeling methods are available for the evaluation of mixing inside a digester [86]. Modeling of the mixing is a natural continuation of e.g. the tracer methods, as previously mentioned, for developing understanding of the complex fluid dynamics in the digester. To model the process, data such as the RTD curve or data from radioactive tracking is useful not only for constructing the model but also for verification of the model's validity before it can be used to develop the process in question. Other types of data collection that can be used to develop models include the experimental methods discussed in Section 3.2. This section addresses the use of computational fluid dynamics (CFD), mass balances and kinetic models in evaluating and improving mixing.

5.1. CFD

The mixing of different anaerobic digesters has been modeled using CFD. CFD is a versatile tool to study the flow fields, velocity contours, turbulence, particle trajectories, movement of dissolved components and for identifying volumes of high mixing intensity and stagnant zones. Like all models, CFD models must be validated against experimental data before they can be relied on to give an accurate representation of the process being studied. However, after validation, a CFD model is a powerful tool to design, evaluate and optimize the process.

The model is built by first constructing the geometry of the studied digester in a computer aided design type program. The geometry is then fitted with a mesh, dividing up the entire volume or domain into smaller cells, and boundary conditions are set for inlets, outlets, walls, etc. The properties of the different phases (gas, liquid and solids) are then defined. Depending on whether the problem is single phase or multiphase, different solvers and turbulence models are selected to calculate how the phase/phases are affected by the geometry and boundary conditions in each individual cell defined by the mesh.

CFD models focus on the fluid dynamics and are separated from the kinetics of e.g. the AD. The fluid dynamics can be used for design, redesign and scale up purposes to produce the optimal mixing conditions. However, this requires knowledge of the effects of mixing on the process and set criteria for optimal mixing. One benefit of CFD modeling is the possibility of presenting visual results, which makes analysis of the results faster, easier and more intuitive. When analyzing the mixing, the flow fields, the direction of the flow and the velocity can be visualized as well as the movement of particles and dissolved components.

5.1.1. Implementation of CFD simulations

CFD models can be used to model digester designs and the design of the mixing and their effects on the mass transfer within the digester. Karim et al. [38] modeled a digester with a gas-lift mixing configuration and evaluated the effect of adding a baffle to the system. Other design changes to the digester were also evaluated for the same setup, e.g. changing the bottom configuration in the digester with the goal of increasing the overall mixing [91]. The models were validated by data from CARPET experiments [34]. Studies of gas-lift mixing by Vesvikar and Al-Dahhan [91] and Karim et al. [34] concluded that the gas flow rate and the positioning and height of the draft tube had little effect on the mixing and volume of stagnant zones in the digester. However, stagnant zones could be reduced by increasing the draft tube diameter and changing to a conical bottom.

CFD models can also be used to simulate very small and detailed areas in the digester as well as the general digester design. Manea and Robescu [55] evaluated the use of different impellers, four and six-blades with tilt angles of 30° and 45°, showing that the number of blades had a marginal effect on the produced flow, while the larger angle impeller produced a higher flow rate inside the draft tube. Meroney and Colorado [60] compared a number of different digester designs with varying numbers of draft tubes and draft tube configurations which all showed good mixing characteristics with few stagnant zones and short circuiting flows.

Wu [96] integrated his mixing model with the biochemical kinetic reactions of the AD process and in doing so bridged the gap between pure CFD models and kinetic models. The model he developed has the ability to trace the production and distribution of a wide range of components including VFA, CO_2 and CH_4 as well as heat transfer, HRT and pH in the entire domain.

A number of studies have been performed and models built to study different aspects of the mixing inside an anaerobic digester using CFD (e.g. [91,32,49,60,50,95,96]). Fig. 1 summarizes a number of CFD studies evaluating different mixing methods, their modeling approaches and validation methods.

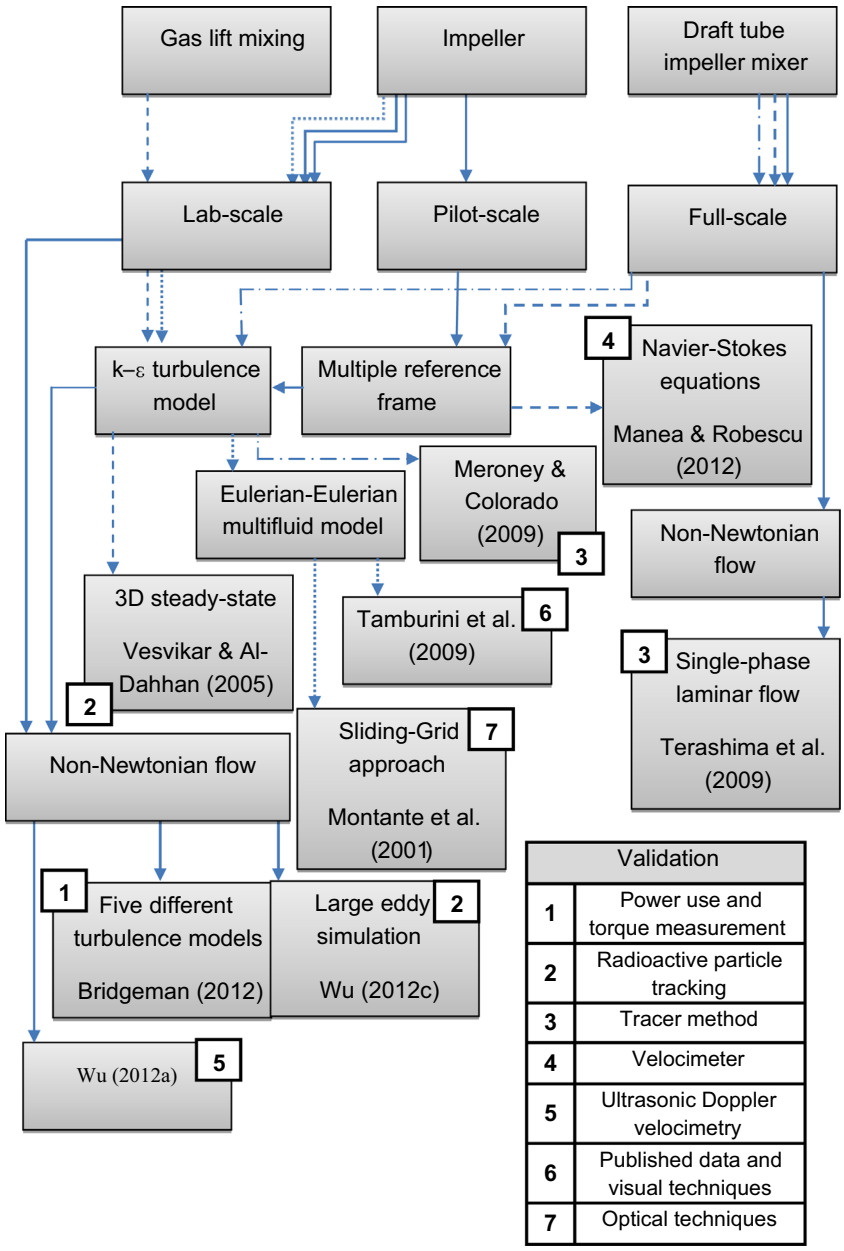

Fig. 1. Nine articles, their modeling approaches and validation methods that examine different types of mixing equipment in lab, pilot and full scale using CFD [11,63,83,85,97].

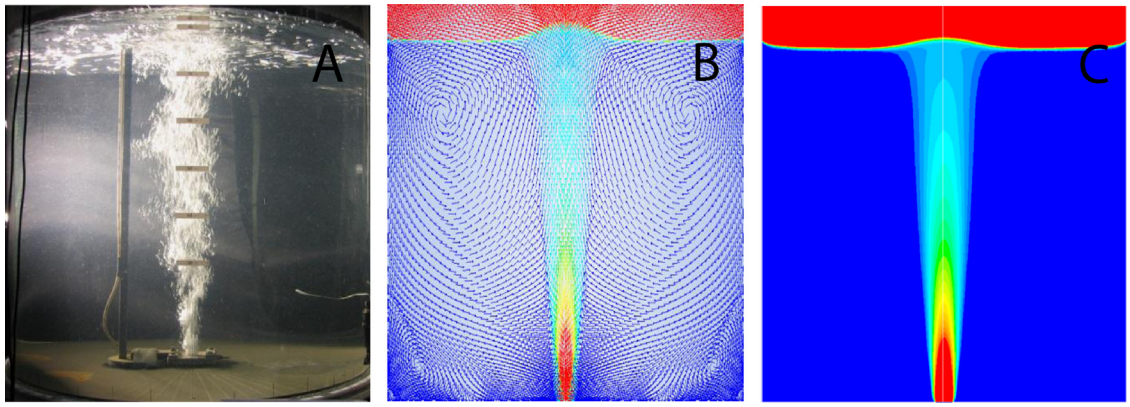

Fig. 2. Simulating the gas flow in a 1 m^3 lab-scale setup. This figure shows a picture of the model setup (A), the liquid velocity field (B) and the gas plume structure (C) at a 15 l/min airflow and 5 mm bubbles. (B) is showing the direction and velocity of the liquid with the red color showing the maximum velocity of 0.34 m/s and the dark blue the lowest velocities. (C) is showing the amount of gas in the digester with the red representing a gas volume fraction above 3% and the dark blue the lowest gas volume fraction.

Fig. 2 shows the example of a simulation of a 1 m^3 lab-scale water model at ABB corporate research. The model is used to evaluate the effects of mixing in an anaerobic digester [7]. The figure shows the gas volume fraction where the maximum value of 3% is at the inlet situated in the bottom middle of the reactor, and the liquid velocity field, showing a maximum liquid velocity of 0.34 m/s. The presented cases were obtained with a air flow rate of 15 l/min. The CFD simulation was performed using the ANSYS Fluent package. The simulation is steady, axisymmetric and based on the Eulerian model where turbulence and bubble induced turbulence are taken into account.

In order to validate the results a series of measurements was taken in the experimental setup. Gas axial velocity and volume fraction were measured using optical probes equipped with double sensors and the liquid velocity was measured using hot wire anemometry. The results from these measurements are compared with the simulation results in Fig. 3.

5.2. Mass balance and kinetic models

Many kinetic models have been created to model the digestion process, of which the Anaerobic Digestion Model No. 1 (ADM1) [5] is the most well-known. Kinetic models describe the biochemical kinetic reactions behavior of the solids, liquids and gases, and in the case of AD the path that the carbohydrates, lipids and proteins take on their way to become biogas. The effects of mixing are rarely addressed in the kinetics and the major rate limiting step is often attributed to hydrolysis. Reuss [68] reported on different model structures to work with the effects of mixing and mass transfer in a bioreactor. Depending on the mixing and its complexity, Reuss listed the two-region mixing model [74], the five-compartment model [66], the mixing-cell model (for multiple impellers) [4], the recycle-backmix circulation model [76], the compartment model [6], and the multicompartment model (three- and two-phase) [67]. The kinetics of the AD process are fairly well known, even though their implementation can be complicated [50], and the mass balance or structure of the models are well developed [68] for the integration of the mixing effects when they have been properly established. Keshtkar et al. [39] modeled the AD of cattle manure using the two-region mixing model and the kinetics presented by Hill [31] and later developed by Angelidaki et al. [2]. The model showed good prediction of experimental data and can be used to simulate reactors with different degrees of mixing. Bello-Mendoza and Sharratt [8] also developed a two-region mixing model for the digestion of sewage sludge. Vavilin and Angelidaki [89] and Vavilin et al. [90] developed 2D and 3D models of the effect of mixing on different microbial populations and methane production, focusing on the inhibiting effects of VFA and the importance of unmixed areas for protecting the methanogens from over acidification (as described in Section 2.1).

6. Results and discussion

We have established in this review that mixing is an important aspect of the AD process. Low intensity mixing is preferable for floc formation and maintaining the juxtapositioning of the microorganisms. However, experiments have shown that the destruction of flocs does not necessarily lead to lower biogas production; hence the importance of flocs in the CSTR is questionable.

The occurrence of process instabilities at startup during higher intensity mixing and continuous mixing is often the reason for lower gas production in the AD experiments studied, but this does not mean that intense mixing has a long term negative effect on the gas production. Lower mixing intensities during startup allow for a more stable process and allow the microbial community to grow. Uneven mixing with protective stagnant zones can work as initiation centers for methanogens and protect them during times of acidification. However, digesters that have previously experienced instabilities also

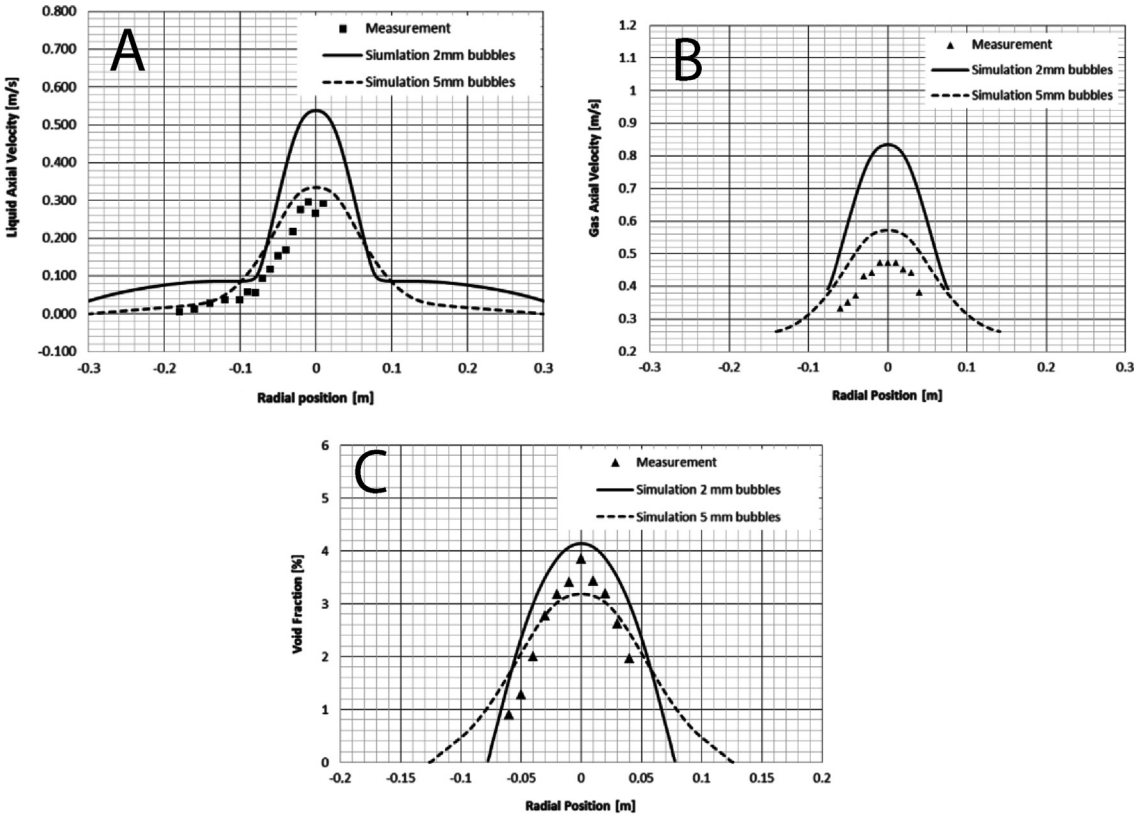

Fig. 3. Comparison between simulation results and experimental measurements. Graphs show the modeled and measured values of the liquid axial velocity (A), the gas axial velocity (B) and the gas volume fraction (C).

show increased resilience against future shock loads caused by a shift in the microbial community. When unmixed digesters perform well it is often attributed to natural mixing in low OLR experiments, and the introduction of mixing has very little effect. The unmixed alternative can produce more biogas than the mixed experiments, but in other experiments it can lead to a 10–20% lower gas production. The question then is of the relative value of additional methane versus the saved energy.

It is very difficult to make general statements about the optimal mixing intensity without power input data, which should be included in the evaluation of a mixing setup, and also because of scale-up issues. According to the continuous mixing experiments evaluated here, mixing intensity had little effect on steady state gas production, and a mixing intensity of 50–100 rpm resulted in a good gas production. The goal of any biogas plant is of course to produce as much methane as possible at the lowest cost/input. In mixing there are three costs to consider, namely the capital cost of the mixing equipment, maintenance cost and operational costs. By reducing mixing intensity and/or reducing the mixing time both operational and maintenance costs can be reduced. In this review the intermittent mixing regime seems to be superior to continuous mixing and shorter mixing periods are preferred both from an energy point of view and for higher gas production. Intermittent mixing can produce identical amounts of biogas to continuous mixing, or it can improve production by lowering costs.

One problem encountered when studying the effect of different mixing regimes in a continuous system is that the removal of solids can be uneven, leading to variations in SRT between digesters. This means that the difference in gas production and reduction rates of different substances can be attributed not only to the direct effect of mixing, but also to the indirect change in SRT. This limits the usefulness of TS, VS and COD reduction since a small or large reduction can equally well be attributed to the mixing as to the breakdown speed of organic material. Great care must be taken with the extraction of effluent when designing experiments to evaluate the effects of mixing. At different mixing levels substrate will stratify differently. Stratification of cattle manure in unmixed reactors for example produces three distinct layers. A floating layer is produced at the surface, a layer of sediment is on the bottom and a layer with a low suspended solids content is in the middle section [73]. Thus, effluent removal from the middle part of a digester is preferred when digesting cattle manure in an unmixed digester, but this makes the comparison between a totally unmixed digester and a continuously mixed digester impossible if the aim is to evaluate the effects of the mixing. These problems can be avoided by mixing all digesters using the same mixing mode immediately before extraction of effluent. This is also why many of the so called unmixed digesters are actually mixed during effluent removal and during feeding to homogenize the content, effectively making it an intermittent mixing mode. Batch assays do not experience the same problems but their usefulness is limited when the results are to be implemented on continuous systems.

The importance of the HRT can be questioned as a simplified way to approximate the SRT if the mixing in the industrial digesters are generally unevenly mixed, especially when sedimentation and/or flotation occurs. When mixing intermittently (or in unmixed digesters) there is also a possibility to remove effluent with lower TS to increase the SRT compared to the HRT. Depending on where the outlet is situated and the substrates tendency to float or sedimentate the SRT can be increased by letting the digester content to stratify before effluent removal from a low TS area of the digester.

The aims of mixing have traditionally been threefold: (i) mixing the new feed with the digester content, (ii) avoiding process problems caused by material buildup in the digester as floating or sedimentating layers, and (iii) heat control. However, as this review has shown, the selected mixing regime has a direct effect on the digestion results and gas yield, and low and intermittent mixing are a valid alternative for keeping the process in operation, as well as having a large effect on the energy balance of the system. There may be reasons to question the use of the CSTR in which these functions are solved by continuously mixing the entire digester content instead of addressing the problems individually.

The evaluation of the effects of mixing is complicated and demands interdisciplinary collaborations between engineers, chemists, microbiologists and hydrodynamic experts to form a complete picture. The evaluations are also very resource intensive, both in time and equipment, and extensive experimental setups with parallel running digesters to evaluate the effects of different mixing intensities and mixing modes in a systematic way are rare. Contradictory results generated from experiments need to be studied at the microbial and chemical level to pinpoint the root causes.

Future research should study mixing from a lower extraction level and examine the system changes continuously with as short a time step as possible (seconds, minutes or hours), depending on the complexity of the analysis needed and the possibility to store samples for later analysis as well as the online measurement equipment. Changes are currently usually studied daily, which may mask important changes in the process. More work should also be performed which includes calculations of the energy efficiency of the mixing, since there are considerable potential energy savings from lowering the mixing intensity and mixing time.

CFD modeling shows considerable promise for increasing understanding of mixing in the AD process but it needs to be linked to the actual production of biogas to a greater extent than presently to determine the effect of e.g. shear forces and stagnant zones on the AD process. These types of extended simulations would be beneficial when designing a digester. Kinetic models of the process including the effects of mixing have been performed to some extent, but still need developing and need to be included as a factor in general kinetic models of the AD process.

Evaluating the effects of mixing in a two-stage AD process by observing the effects of mixing on the individual process stages, hydrolysis/fermentation and methanogenesis, would help to increase understanding of mixing. The unmixed digester emulates the natural process with more or less stagnant volumes of water which might produce a stable AD, but is far from optimized for an industrial AD system. Intense mixing on the other hand can make the process unstable, hypothetically caused by an uneven effect of mixing between hydrolysis/fermentation and methanogens or slower adaptation and growth of the methanogens.

The experimental setups reviewed here, compiled in Figs. A1 and A2 and Tables A1 and A2 in the Appendix A will hopefully support other researchers in their endeavors to optimize the mixing in the AD process. The contradicting results presented by some researchers are of course of great interest because the solution is likely to lie in the problems and the details.

7. Conclusions

The anaerobic digestion process is a highly adaptable process and the microbiological community will develop to handle most environmental changes including higher mixing intensities. However, during rapid changes a lower mixing level can give a more stable process. Laboratory data needs to be analyzed for digester design and operation not only for evaluating the effects on the gas production but also for determining why the mixing effects the gas production. There are a number of tools available to study the fluid dynamics and mass transfer within laboratory and full scale digesters that can support validation of laboratory experiments for full scale digester conditions, including CFD and laboratory tracer methods. Different mixing regimes affects the flow fields, turbulence and shear stress and the

connection between the mixing and the disrupting effect on the biogas process needs to be studied further on a microbial and chemical level. The root causes to the effects seen during different mixing regimes are still not well established.

Some authors claim that mixing affects the balance between hydrolysis/fermentation and methanogenesis during startup, causing an accumulation of VFA that inhibits the methanogens. A lower degree of mixing or stagnant zones can be beneficial during the startup phase to allow for methanogenic biomass growth before an increase in the mixing intensity takes place. Experiments of mixing using the two-stage AD process could help develop the theory in this area. There is a need for some standardization of experimental design to study the effects of mixing. The mixing mode and mixing intensity of all digesters should be the same during effluent removal to obtain the same representative SRT, independently of the mixing mode that is being evaluated. Steady state gas production values should also be more widely measured since the instabilities of the AD experiments are often experienced during startup, at higher mixing intensities, which is a negligible timespan in the lifetime of an industrial digester. Rather than studying a wider range of mixing equipment, regimes and intensities, the focus of further experiments should be to verify previous results and focus on new and inventive ways to study and increase the understanding of the direct effects and symptoms caused by the different mixing regimes on the anaerobic process.

Optimization is not only about increasing biogas production. There is also an opportunity to reduce the energy demand as well as operational and maintenance costs by reducing the mixing. Intermittent mixing has been shown to be able to produce the same amount of biogas and even improve gas production compared to a continuously mixed system while decreasing the maintenance and energy demand of the process. It is reasonable to challenge the digester design and the wide use of the continuously stirred tank reactor.

Appendix A

See Figs. A1 and A2 and Tables A1 and A2.

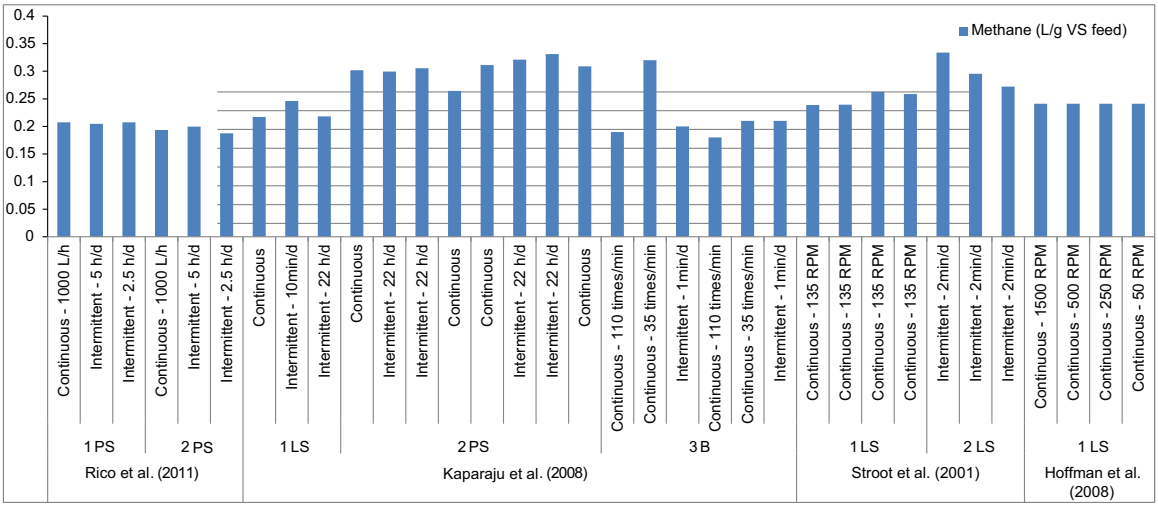

Fig. A1. This figure, together with Fig. A2, presents the methane yield of a number of mixing experiments, showing the differences between the mixing modes chosen. LS, PS and B stand for Lab-Scale, Pilot-Scale and Batch assays respectively and the numbers refer to different experiments in the series. The figure should be read in conjunction with Table A1 for detailed descriptions of the experiments. It should be noted that Kaparaju et al. [33]. 2PS is a single digester series but mixing was continuously changed between feeding batches and should be read as pairs of two from left to right. Stroot et al. [80] 1 LS was performed with different inoculumns and 2 LS was compared to continuous mixing but no methane values were presented, details are presented in Tables A1 and A2.

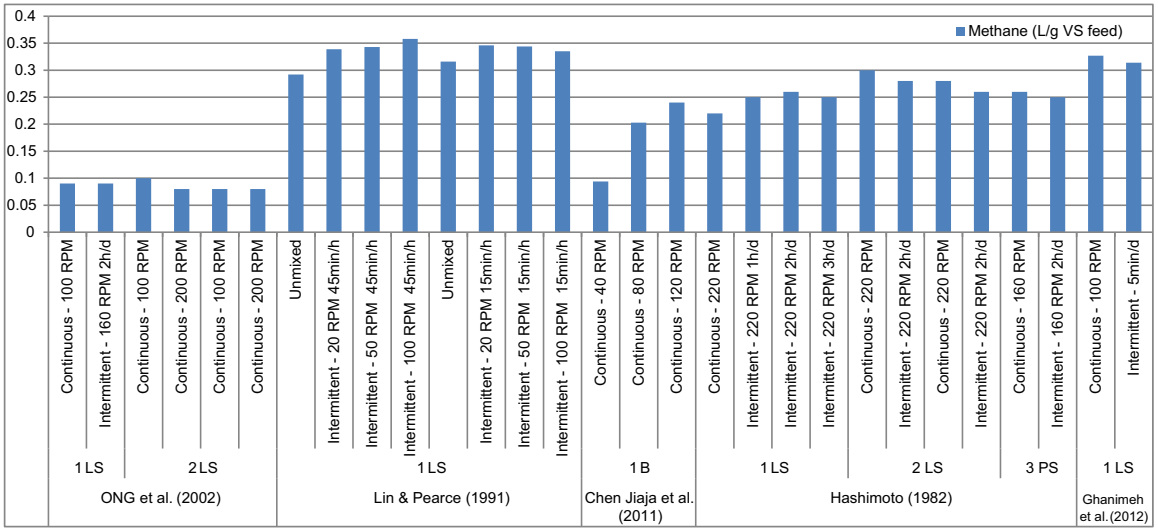

Fig. A2. This figure, together with Fig. A1, presents the methane yield of a number of mixing experiments showing the differences between the mixing modes chosen. LS, PS and B stand for Lab-Scale, Pilot-Scale and Batch assays respectively and the numbers refer to different experiments in the series. The figure should be read in conjunction with Table A1 for detailed descriptions of the experiments. It should be noted that [48] 1 LS is a single digester series but the intermittent mixing was changed after the first set of experiments to reduce the mixing period (4+4) and that the methane yield is presented as L/g TCOD removed. Ong et al. [65] 2LS tested two different positions of the two impellers so that they should be viewed as 2+2.

Table A1
Experimental setups for the evaluation of mixing.

Reference		Temp (°C)	Mixing mode	Details of mixing	Feeding mode	Inoculum	Feed type
Karim et al. [37]							
3.73 L	1 Lab-Scale	35	Unmixed		Alternate days	AD sludge, cow manure	Screened cow manure
			Biogas-mixed	1 L/min			
			Impeller-mixed	275 rpm, 62 mm diameter			
			Slurry recirculation	0.82 L/min			
3.73 L	2 Lab-Scale	35	Unmixed		Alternate days	AD sludge, cow manure	Screened cow manure
			Biogas-mixed	1 L/min			
			Impeller-mixed	275 rpm, 62 mm diameter			
			Slurry recirculation	0.82 L/min			
3.73 L	3 Lab-Scale	35	Unmixed		Alternate days	AD sludge, cow manure	Screened cow manure
			Biogas-mixed	1 L/min			
			Impeller-mixed	275 rpm, 62 mm diameter			
Rico et al. [69]							
1500 L	1 Pilot-scale	37	Continuous	1000 L/h pump	30 min 5 times/d	Screened dairy manure	Screened dairy manure
6 month in opperation			Intermittent	30 min 10 times/d			
before experiment start			Intermittent	automatic for heating 2.5 h/d			
	2 Pilot-scale	37	Continuous	1000 L/h pump	30 min 10 times/d	Screened dairy manure	Screened dairy manure
			Intermittent	30 min 10 times/day			
			Intermittent	automatic for heating 2.5 h/d			
Kaparaju et al. [33][a]							
3.6 L	1 Lab-Scale	55	Continuous	Stirrer	12 h intervall	AD sludge (pig+cow manure)	Blended cow manure
			Intermittent	10 min before extraction/feed			
			Intermittent	no mixing 2 h before ext./feed			
500 L	2 Pilot-scale	54 ± 1	Continuous	Stirrer, 5 min on/off, 2 impellers	8 h intervall	AD sludge (pig+cow manure)	Cow manure
	Mixing regim 1		Intermittent	No mixing 2 h before wasting/ feeding			
	Mixing regim 2		Intermittent	Feed batch 2			
			Continuous				
	Mixing regim 3		Continuous	Feed batch 3			
			Intermittent				
	Mixing regim 4		Intermittent	Feed batch 4			
			Continuous				
0.4 L	3 Batch assays	55	Continuous	110 times/min, 3.5 cm stroke, 10/90	n.a.	AD sludge (pig+cow manure)	Cow manure
Substrate/ inoculumn			Continuous	35 times/min, 1.2 cm stroke, 10/90	n.a		
			Intermittent	1 min before sampleing, 10/90	n.a.		
			Continuous	110 times/min,3.5 cm stroke, 40/60	n.a.		
			Continuous	35 times/min,1.2 cm stroke, 40/60	n.a		
			Intermittent	1 min before sampleing, 40/ 60	n.a.		
Stroot et al. [80][a]							
1 L	1 Lab-scale	37	Continuous/ minimal	135 rpm (est.)/2 min (see Lab-scale 2)	n.a.	No exogenous inoculum	PS+WAS
Continuous for 14 days			Continuous/ minimal	135 rpm (est.)/2 min (see Lab-scale 2)	n.a.	Cattle manure and AD sludge 25/75	
Then minimal			Continuous/ minimal	135 rpm (est.)/2 min (see lab-scale 2)	n.a.	Cattle manure	
			Continuous/ minimal	135 rpm (est.)/2 min (see Lab-scale 2)	n.a.	Anaerobic sludge	
1 L	2 Lab-scale	37	Continuous	135 rpm (est.)	Stop during overload	Anaerobic sludge	PS+WAS
	Mixing regim 1		Continuous	135 rpm (est.)			PS+WAS**t**
			Continuous (A)	135 rpm (est.)			PS**t**+WAS**t**
			Minimal (B)	1 min before wasting, 1 min after feed	n.a.		PS+WAS
			Minimal	1 min before wasting, 1 min after feed	n.a.		PS+WAS**t**
			Minimal	1 min before wasting, 1 min after feed	n.a.		PS**t**+WAS**t**
0.5 L	3 Lab-scale	37	Continuous (A)	135 rpm (est.)	Stop during overload	Digester A, experiment 2	PS**t**+WAS**t**
	Mixing regim 2		Minimal (A)	1 min before wasting, 1 min after feed		Digester A, experiment 2	
			Continuous (B)	135 rpm (est.)		Digester B, experiment 2	PS+WAS

Table A1 (*continued*)

Reference		Temp (°C)	Mixing mode	Details of mixing	Feeding mode	Inoculum	Feed type
			Minimal (B)	1 min before wasting, 1 min after feed		Digester B, experiment 2	
Sulaiman et al. [81]							
500.000 L	1 Full-Scale	36 ± 2	Natural		every 6 h	n.a	Palm oil mill effluent
			Intermittent	pumping 30 min every 6 h, Horiz. inlet		n.a	
			Intermittent	30 min every 6 h, Horiz. & vertical inlet		n.a	
			Intermittent	30 min every 2 h, Horiz. & vertical inlet		n.a	
[32]							
4.5 L 62-mm axial impeller	1 Lab-scale	34 ± 1	Continuous Continuous	1500 rpm 500 rpm	every 24 ± 1 h	AD sludge, waste water treatment	Screened dairy manure
			Continuous Continuous	250 rpm 50 rpm			
Gomez							
3 L	1 Lab-scale	37[b]	HM > LM > SC HM > LM > SC HM > LM > SC HM > LM > SC	HM=high mixing 200 rpm LM=low mixing 80 rpm SC=static condition No mixing LM & SC 200 RPM bef. Feed. and after wast.	n.a n.a n.a n.a	AD sludge, waste water treatment	PS PS and FVFMSW
Karim et al. [36]							
3.73 L	1 Lab-scale	35 ± 2	Biogas-mixed Biogas-mixed Impeller-mixed Slurry recirculation	1 L/min (60° hopper bottom) 1 L/min (25° hopper bottom) 275 rpm, 62 mm diameter (25° hopper bottom) 0.82 L/min (25° hopper bottom)	Alternate days	AD sludge, cow manure	Screened cow manure
3.73 L	2 Lab-scale		Unmixed Impeller-mixed	(25° HB) 275 rpm, 62 mm diameter (25° HB)	Alternate days	AD sludge, cow manure	Screened cow manure
3.73 L	3 Lab-scale		Unmixed Biogas-mixed Impeller-mixed Slurry recirculation	(25° HB) 1 L/min (25° hopper bottom) 275 rpm, 62 mm diameter (25° hopper bottom) 0.82 L/min (25° hopper bottom)	Alternate days	AD sludge, cow manure	Screened cow manure
Karim et al. [35]							
3.73 L	1 Lab-scale	35 ± 2	Unmixed Continuous Continuous Continuous Continuous Continuous	Draft tube 40 mm from bottom Biogas-mixed 1 L/min Biogas-mixed 1 L/min Biogas-mixed 2 L/min Biogas-mixed 3 L/min Biogas-mixed 1 L/min	Alternate days	AD sludge, cow manure	Screened cow manure
Ong et al. [65]							
10 L	1 Lab-scale	35 ± 1	Continuous Intermittent	100 rpm 4 × 30 min/day 160 rpm	0.042 L per 15 min 0.25 L 4 times/day	AD sludge, cow manure	Cattle manure (reconstituted)
	2 Lab-scale		Continuous Continuous Continuous Continuous	POS I[c], 100 rpm POS I[c], 200 rpm POS II[c], 100 rpm POS II[c], 200 rpm	n.a. n.a. n.a. n.a.	AD sludge, cow manure	Cattle manure (reconstituted)
Kim et al. [41][a]							
1.1 L (0.8 L active)	1 Lab-scale	55	Batch-fed CSTR Continuously fed CSTR	Magnetic stirrer, visually homogenius Magnetic stirrer	n.a. n.a.	AD sludge	Dog food
0.5 L (0.12 L active)			two-phase system batch-fed unmixed	p1 manually, p2 magnetic stirrer Manually before wasting & after feeding	n.a. n.a.		
1.1 L (0.8 L active)	2 Lab-scale	35	batch-fed CSTR Continuously fed CSTR	Magnetic stirrer Magnetic stirrer	n.a. n.a.	AD sludge	Dog food
0.5 L (0.12 L active)			Two-phase system Batch-fed unmixed	p1 manually, p2 magnetic stirrer Manually before wasting & after feeding	n.a. n.a.		
Rojas et al. [71]							
0.5 L (0.4 active)	1 Batch assays	37	Continuous	60 rpm magnetic stirrer	n.a.	AD sludge, manure & kitchen waste	None

Table A1 (*continued*)

Reference		Temp (°C)	Mixing mode	Details of mixing	Feeding mode	Inoculum	Feed type
			Unmixed		n.a.		
			Continuous	60 rpm magnetic stirrer	n.a.		0.01 L lipid-rich
			Unmixed		n.a.		
			Continuous	60 rpm magnetic stirrer	n.a.		5 g corn silage
			Unmixed		n.a.		
7 L	2 Lab-scale	37	Continuous	60 rpm impeller	Every 2 days	AD sludge, manure & kitchen waste	
			Unmixed				
0.5 L (0.4 active)	3 Batch assays	37	Continuous	60 rpm magnetic stirrer	n.a.	AD sludge & Manure 2/3	None
			Unmixed		n.a.		
			Continuous	60 rpm magnetic stirrer	n.a.		0.01 L lipid-rich
			Unmixed		n.a.		
			Continuous	60 rpm magnetic stirrer	n.a.		5 g corn silage
			Unmixed		n.a.		
Lin and Pearce [48]							
7.0 L	1 Lab-scale Mixing regim 1	20	Unmixed		1 min/h	AD sludge, potato-processing	Primary clarifier influent, potato
			Intermittent	Impeller mixing 45 min/h 20 rpm			
			Intermittent	Impeller mixing 45 min/h 50 rpm			
			Intermittent	Impeller mixing 45 min/h 100 rpm			
	1 Lab-scale Mixing regim 2		Unmixed		1 min/h	AD sludge, potato-processing	Primary clarifier influent, potato
			Intermittent	Impeller mixing 15 min/h 20 rpm			
			Intermittent	Impeller mixing 15 min/h 50 rpm			
			Intermittent	Impeller mixing 15 min/h 100 rpm			
Hamdi et al. [28]							
	1 Batch assays	35	Unmixed	0 rpm shaker	n.a.	AD sludge, olive mill wastewater OMW	Unmodified OMW
			Continuous	20 rpm shaker	n.a.		
			Continuous	50 rpm shaker	n.a.		
			Continuous	200 rpm shaker	n.a.		
	2 Batch assays	35	Unmixed	0 rpm shaker	n.a.	AD sludge, olive mill wastewater OMW	Fermented OMW
			Continuous	20 rpm shaker	n.a.		
			Continuous	50 rpm shaker	n.a.		
			Continuous	200 rpm shaker	n.a.		
Chen Jiaja et al. [18]							
90 L	1 Batch assays	35	Continuous	40 rpm	n.a.	AD sludge, waste water treatment	Rice straw
			Continuous	80 rpm	n.a.		
			Continuous	120 rpm	n.a.		
Hashimoto [30]							
4 L (3 L active volume)	1 Lab-scale	55 ± 1	Continuous	220 rpm	n.a.	AD sludge, pilot plant	Beef cattle manure
			Intermittent	220 rpm 1 h/d	n.a.		
			Intermittent	220 rpm 2 h/d	n.a.		
			Intermittent	220 rpm 3 h/d	n.a.		
4 L (3 L active volume)	2 Lab-scale		Continuous	220 rpm	n.a.	AD sludge, pilot plant	Beef cattle manure
			Intermittent	220 rpm 2 h/d	n.a.		
			Continuous	220 rpm	n.a.		
			Intermittent	220 rpm 2 h/d	n.a.		
5700 L	4 Pilot-scale		Continuous	160 rpm	n.a.	AD sludge, pilot plant	beef cattle manure
			Intermittent	160 rpm 2 h/d	n.a.		
Kowalczyk et al. [43,44]							
	1 Lab-scale	38	Intermittent	2 h stirring/1 h break	1 s every day	AD-sludge	CCM, CM + trace elements
			Intermittent	7 h stirring/1 h break			
			Continuous	68–71 rpm for all digesters			
	2 Lab-scale	38	Intermittent	10 min stirring/230 min break	1 s every day	AD-sludge	MS
			Intermittent	10 min stirring/50 min break			
			Continuous	68–71 rpm for all digesters			
Ghanimeh et al. [24]							
14 L (9 L active volume)	1 Lab-scale	55	Continuous	100 rpm	n.a.	Fresh manure	SOFMSW
	Startup		Intermittent	Few minutes before wasting & after feed	n.a.		
Lindmark et al. [51]							
1 L (0.7 L active volume)	1 Batch assays	33	Continuous	150 rpm shaker	–	AD-sludge, SOFMSW	SOFMSW
			Continuous	25 rpm shaker	–	AD-sludge, SOFMSW	SOFMSW
			Intermittent	Less than 1 min/day	–	AD-sludge, SOFMSW	SOFMSW
		33	Continuous	150 rpm shaker	–	AD-sludge, SOFMSW	Inoculum only

Table A1 (*continued*)

Reference		Temp (°C)	Mixing mode	Details of mixing	Feeding mode	Inoculum	Feed type
1 L (0.7 L active volume)	2 Batch assays						
			Continuous	25 rpm shaker	–	AD-sludge, SOFMSW	Inoculum only
			Intermittent	Less than 1 min/day	–	AD-sludge, SOFMSW	Inoculum only

n.a.—Not available.

[a] Data also gained by personal communications with the authors.
[b] Only wrote mesophilic without temperature interval.
[c] Impeller position, POS I: equidistance from top and bottom, POS II: one impellerr 1/3 from top, other 1/5 from bottom.

Table A2
Experimental setups for the evaluation of mixing.

Reference	Mixing mode	HRT (days)	OLR (g TS/L d)	OLR (g VS/L d)	biogas production (L/L d)	biogas production (L/g VS feed)	Methane yield (L/g VS feed)	Methane content (%)
Karim et al. [37]								
3.73 L	Unmixed	16.2	3.1	2	0.84 ± 0.07	0.42[a]	0.27	64 ± 3
	Biogas-mixed	16.2	3.1	2	0.94 ± 0.07	0.47[a]	0.26	56 ± 3
	Impeller-mixed	16.2	3.1	2	0.88 ± 0.09	0.44[a]	0.27	61 ± 3
	Slurry recirculation	16.2	3.1	2	0.85 ± 0.09	0.425[a]	0.28	67 ± 2
3.73 L	Unmixed	16.2	6.2	3.2	0.92 ± 0.1	0.29[a]	0.19	66 ± 3
	Biogas-mixed	16.2	6.2	3.2	1.07 ± 0.08	0.33[a]	0.21	65 ± 4
	Impeller-mixed	16.2	6.2	3.2	1.14 ± 0.13	0.36[a]	0.23	65 ± 3
	Slurry recirculation	16.2	6.2	3.2	1.20 ± 0.14	0.38[a]	0.24	66 ± 4
3.73 L	Unmixed	16.2	9.3	4.7	1.13 ± 0.14	0.24[a]	0.15	0.64
	Biogas-mixed	16.2	9.3	4.7	1.64 ± 0.32[b]	0.35[ab]	0.23[b]	0.66
	Impeller-mixed	16.2	9.3	4.7	1.25 ± 0.12	0.27[a]	0.17	0.63
[69]								
1500 L	Continuous	20	n.a	2.3	0.71	0.31[a]	0.21[a]	67.2–70.2
6 month in opperation	Intermittent	20	n.a	2.3	0.70	0.30[a]	0.20[a]	67.2–70.2
Before experiment start	Intermittent	20	n.a	2.3	0.71	0.31[a]	0.21[a]	67.2–70.2
	Continuous	10	n.a	4.5	1.30	0.29[a]	0.19[a]	67
	Intermittent	10	n.a	4.5	1.34	0.30[a]	0.20[a]	67
	Intermittent	10	n.a	4.5	1.26	0.28[a]	0.19[a]	67
[33]								
3.6 L	Continuous	15	5.2	4.0	0.67 ± 0.05[a]	0.339[a]	0.217 ± 0.032	64.1
	Intermittent	15	5.2	4.0	0.75 ± 0.10[a]	0.384[a]	0.246 ± 0.031	64.1
	Intermittent	15	5.2	4.0	0.68 ± 0.08[a]	0.346[a]	0.218 ± 0.030	63.0
500 L	Continuous	20	3.5[a]	3.0[a]	1.198	0.435	0.435	69.4
	Intermittent	20	3.5[a]	3.0[a]	1.206	0.446	0.446	67.1
	Intermittent	20	3.5[a]	3.0[a]	1.144	0.432	0.432	70.7
	Continuous	20	3.5[a]	3.0[a]	0.994	0.377	0.377	70.1
	Continuous	20	3.5[a]	3.0[a]	1.08	0.451	0.451	69.0
	Intermittent	20	3.5[a]	3.0[a]	1.1	0.465	0.465	69.0
	Intermittent	20	3.5[a]	3.0[a]	1.256	0.477	0.477	69.4
	Continuous	20	3.5[a]	3.0[a]	1.204	0.442	0.442	69.9
			g TS/L	**g VS/L**				
0.4 L	Continuous	–	8.1[a]	6.2[a]	n.a.	n.a.	0.19	n.a.
Substrate/inoculumn	Continuous	–	8.1[a]	6.2[a]	n.a.	n.a.	0.32	n.a.
	minimal intermittent	–	8.1[a]	6.2[a]	n.a.	n.a.	0.20	n.a
	Continuous	–-	32.4[a]	24.8[a]	n.a.	n.a.	0.18	n.a
	Continuous	–	32.4[a]	24.8[a]	n.a.	n.a.	0.21	n.a
	Minimal intermittent	–	32.4[a]	24.8[a]	n.a.	n.a.	0.21	n.a
Stroot et al. [80]								
1 L	Continuous/ minimal	19.8	n.a.	3.7	1.59	0.43	0.24[a]	55.5
Continuous for 14 days	Continuous/ minimal	19.6	n.a.	3.7	1.65	0.44	0.24[a]	54.4
Then minimal	Continuous/ minimal	20.3	n.a.	3.7	1.69	0.47	0.26[a]	55.9
	Continuous/ minimal	20.0	n.a.	3.7	1.74	0.47	0.26[a]	55.0
1 L	Continuous	n.a.	n.a.	3.5	0.1[cd]	n.a.	n.a.	n.a.
	Continuous	n.a	n.a.	7.6	0.1[cd]	n.a.	n.a.	n.a.
	Continuous (A)	n.a.	n.a.	9.4	0.25[cd]	n.a.	n.a.	n.a
	Minimal (B)	20.5	n.a	3.5	1.91	0.56	0.33[a]	59.6
	Minimal	18.8	n.a	7.6	4.22	0.52	0.30[a]	56.8
	Minimal	16.8	n.a.	9.4	5.49	0.49	0.27[a]	55.5

Table A2 (*continued*)

Reference	Mixing mode	HRT (days)	OLR (g TS/L d)	OLR (g VS/L d)	biogas production (L/L d)	biogas production (L/g VS feed)	Methane yield (L/g VS feed)	Methane content (%)
0.5 L	Continuous (A)	n.a.	n.a.	9.4	n.a.	n.a.	n.a.	n.a.
	Minimal (A)	n.a.	n.a.	9.4	n.a.	n.a.	n.a.	n.a.
	Continuous (B)	n.a.	n.a.	3.5	n.a.	n.a.	n.a.	n.a.
	Minimal (B)	n.a.	n.a.	3.5	n.a	n.a.	n.a.	n.a.
Sulaiman et al. [81]			**g COD/L d**					
500.000 L	Unmixed	10	3.9–7.8	n.a	2.1 ± 0.3	n.a.	n.a.	48[a]
	Intermittent	10	2.5–7.8	n.a.	2.5 ± 0.4	n.a.	n.a.	56[a]
	Intermittent	10	3.1–7.5	n.a.	2.1 ± 0.1	n.a.	n.a.	52[a]
	Intermittent	10	4.0–8.5	n.a.	0.9 ± 0.4	n.a.	n.a.	44[a]
Hoffman et al. [32]								
4.5 L	Continuous	83–15	n.a.	0.6–3.5	n.a.	n.a.	0.241 ± 0.007	67.4 ± 5.0
	Continuous	83–15	n.a.	0.6–3.5	n.a.	n.a.	0.241 ± 0.007	67.4 ± 5.0
	Continuous	83–15	n.a.	0.6–3.5	n.a.	n.a.	0.241 ± 0.007	67.4 ± 5.0
	Continuous	83–15	n.a.	0.6–3.5	n.a.	n.a.	0.241 ± 0.007	67.4 ± 5.0
Gomez								
3 L	HM > LM > SC	47–37	n.a.	0.83–1.2	0.2–0.3	0.2–0.4	n.a.	n.a.
	HM > LM > SC	47–37	n.a.	0.83–1.2	0.3–0.5	0.3–0.5	n.a.	n.a.
	HM > LM > SC	47–37	n.a.	0.83–1.43	0.2–1.5	0.3–0.6	n.a.	n.a.
	HM > LM > SC	47–37	n.a.	0.83–1.43	0.3–1.2	0.3–0.6	n.a.	n.a.
Karim et al. [36]								
3.73 L	Biogas-mixed	16.2	3.08	2.0	0.84 ± 0.1	0.42 ± 0.05[a]	0.26 ± 0.03	62 ± 3
	Biogas-mixed	16.2	3.08	2.0	0.94 ± 0.07	0.47 ± 0.04[a]	0.26 ± 0.02	56 ± 3
	Impeller-mixed	16.2	3.08	2.0	0.88 ± 0.09	0.44 ± 0.05[a]	0.27 ± 0.03	61 ± 3
	Slurry recirculation	16.2	3.08	2.0	0.85 ± 0.09	0.43 ± 0.05[a]	0.28 ± 0.03	67 ± 2
3.73 L	Unmixed	16.2	3.08	2.0	0.84 ± 0.07	0.42 ± 0.04[a]	0.27 ± 0.02	64 ± 3
	Impeller-mixed	16.2	3.08	2.0	0.93 ± 0.09	0.47 ± 0.05[a]	0.31 ± 0.03	66 ± 2
3.73 L	Unmixed	16.2	6.2	3.24	0.92 ± 0.1	0.28 ± 0.03[a]	0.19 ± 0.02	66 ± 3
	Biogas-mixed	16.2	6.2	3.24	1.07 ± 0.08	0.33 ± 0.03[a]	0.21 ± 0.02	65 ± 4
	Impeller-mixed	16.2	6.2	3.24	1.14 ± 0.13	0.35 ± 0.04[a]	0.23 ± 0.03	65 ± 3
	Slurry recirculation	16.2	6.2	3.24	1.20 ± 0.14	0.37 ± 0.04[a]	0.24 ± 0.03	66 ± 4
Karim et al. [35]				**g TCOD/L d**				
3.73 L	Unmixed	16.2	3.08	3.37	0.68 ± 0.027	n.a.	n.a.	67 ± 0.1
	Continuous	16.2	3.08	3.37	0.67 ± 0.013	n.a.	n.a.	66 ± 0.2
	Continuous	16.2	3.08	3.37	0.69 ± 0.025	n.a.	n.a.	65 ± 0.6
	Continuous	16.2	3.08	3.37	0.60 ± 0.023	n.a.	n.a.	66 ± 0.3
	Continuous	16.2	3.08	3.37	0.61 ± 0.022	n.a.	n.a.	67 ± 0.6
	Continuous	16.2	3.08	3.37	0.65 ± 0.046	n.a.	n.a.	66 ± 0.3
Ong et al. [65]								
10 L	Continuous	10	8	7.2	1.39[a]	0.2	0.09[a]	45.2
	Intermittent	10	8	7.2	1.36[a]	0.2	0.09[a]	46.0
	Continuous	10	8	7.2	1.62[a]	0.23[a]	0.10[a]	45.7
	Continuous	10	8	7.2	1.30[a]	0.18[a]	0.08[a]	45.7
	Continuous	10	8	7.2	1.33[a]	0.18[a]	0.08[a]	46.0
	Continuous	10	8	7.2	1.31[a]	0.18[a]	0.08[a]	45.2
[41]								
1.1 L (0.8 L active)	batch-fed CSTR	20	2[a]	1.8[a]	0.70[a]	0.391 ± 0.037	n.a.	n.a.
	continuously fed CSTR	20	2[a]	1.8[a]	0.83[a]	0.461 ± 0.029	n.a.	n.a.
Phase one 0.5 L (0.12 L active)	Two-phase system	2 + 18	2[a]	1.8[a]	1.09[a]	0.607 ± 0.037	n.a.	n.a.
	Batch-fed unmixed	20	2[a]	1.8[a]	1.29[a]	0.716 ± 0.042	n.a.	n.a.
1.1 L (0.8 L active)	Batch-fed CSTR	20	2[a]	1.8[a]	0.89[a]	0.494 ± 0.032	n.a.	n.a.
	Continuously fed CSTR	20	2[a]	1.8[a]	0.87[a]	0.482 ± 0.036	n.a	n.a
Phase one 0.5 L (0.12 L active)	Two-phase system	2 + 18	2[a]	1.8[a]	0.96[a]	0.531 ± 0.057	n.a	n.a
	Batch-fed unmixed	20	2[a]	1.8[a]	1.00[a]	0.556 ± 0.037	n.a	n.a
[71]			**g TS**	**g VS**	**L/g VS d (Maximum)**			
0.5 L (0.4 active)	Continuous	–	n.a.	n.a.	n.a	n.a.	n.a.	n.a.
	Unmixed	–	n.a.	n.a.	n.a	n.a.	n.a.	n.a.
	Continuous	–	1.35[a]	1.05[a]	0.400[c]	0.699	n.a.	n.a.
	Unmixed	–	1.35[a]	1.05[a]	0.250[c]	0.318	n.a.	n.a.
	Continuous	–	1.53[a]	1.46[a]	0.130[c]	0.437	n.a.	n.a.
	Unmixed	–	1.53[a]	1.46[a]	0.050[c]	0.175	n.a.	n.a.
					L/g VS d (Maximum)			
7 L	Continuous	93[a]	0.72[a]	0.58[a]	0.740[c]	1.28[a]	n.a.	n.a.
	Unmixed	93[a]	0.72[a]	0.58[a]	0.425[a]	0.73[a]	n.a.	n.a.
			g TS	g VS	**L/g VS d (maximum)**			
0.5 L (0.4 active)	Continuous	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Table A2 (*continued*)

Reference	Mixing mode	HRT (days)	OLR (g TS/L d)	OLR (g VS/L d)	biogas production (L/L d)	biogas production (L/g VS feed)	Methane yield (L/g VS feed)	Methane content (%)
	Unmixed	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Continuous	–	1.35[a]	1.05[a]	0.300[c]	0.700	n.a.	n.a.
	Unmixed	–	1.35[a]	1.05[a]	0.250[c]	0.700	n.a	n.a
	Continuous	–	1.55[a]	1.49[a]	0.185	0.400[c]	n.a	n.a
	Unmixed	–	1.55[a]	1.49[a]	0.125	0.500[c]	n.a	n.a
[48]				**gTCOD/Ld**		**L biogas/g TCOD removed**	**L CH_4/g TCOD removed**	
7.0 L	Unmixed	7		0.5	n.a	0.40[a]	0.292	73.5
	Intermittent	7		0.5	n.a.	0.44[a]	0.339	76.5
	Intermittent	7		0.5	n.a.	0.45[a]	0.343	76.4
	Intermittent	7		0.5	n.a.	0.47[a]	0.358	75.5
				gTCOD/Ld		**L biogas/g TCOD removed**	**L CH4/g TCOD removed**	
	Unmixed	7		0.5	n.a.	0.43[a]	0.316	73.5
	Intermittent	7		0.5	n.a.	0.45[a]	0.346	76.5
	Intermittent	7		0.5	n.a.	0.45[a]	0.344	76.4
	Intermittent	7		0.5	n.a.	0.44[a]	0.335	75.5
[28]				**g COD/L**				
	Unmixed	–		20	n.a.	n.a.	n.a.	n.a.
	Continuous	–		20	n.a.	n.a.	n.a.	n.a.
	Continuous	–		20	n.a.	n.a.	n.a.	n.a.
	Continuous	–		20	n.a.	n.a.	n.a.	n.a.
				g COD/L				
	Unmixed	–		20	n.a.	n.a.	n.a.	n.a.
	Continuous	–		20	n.a.	n.a.	n.a.	n.a.
	Continuous	–		20	n.a.	n.a.	n.a.	n.a.
	Continuous	–		20	n.a.	n.a.	n.a.	n.a.
[18]			**g TS/L**	**g VS/L**				
90 L	Continuous	-	62.1	53.3	n.a.	0.2183	0.094[a]	43[a]
	Continuous	-	62.1	53.3	n.a.	0.3778	0.203[a]	54[a]
	Continuous	-	62.1	53.3	n.a.	0.4516	0.240[a]	53[a]
[30]								
4 L (3 L active volume)	Continuous	6	n.a.	9.6	n.a.	0.45[a]	0.22	49.4 ± 3.0
	Intermittent	6	n.a.	9.6	n.a.	0.50[a]	0.25	49.7 ± 3.3
	Intermittent	6	n.a.	9.6	n.a.	0.53[a]	0.26	49.5 ± 2.9
	Intermittent	6	n.a.	9.6	n.a.	0.51[a]	0.25	49.2 ± 3.4
4 L (3 L active volume)	Continuous	6	n.a.	n.a.	n.a.	0.53[a]	0.30 ± 0.01	56.2 ± 0.2
	Intermittent	6	n.a.	n.a.	n.a.	0.50[a]	0.28 ± 0	56.1 ± 0.5
	Continuous	4	n.a.	n.a.	n.a.	0.50[a]	0.28 ± 0.01	56.4 ± 0.4
	Intermittent	4	n.a.	n.a.	n.a.	0.47[a]	0.26 ± 0.02	55.6 ± 0.2
5700 L	Continuous	6	n.a.	n.a.	n.a.	0.50	0.26	52.5 ± 0.8
	Intermittent	6	n.a.	n.a.	n.a.	0.46	0.25	53.9 ± 4.7
Kowalczyk et al. [43,44]					L/h			
	Intermittent	n.a.	n.a.	1.6–4	1.3[c]	n.a.	n.a.	50-60[c]
	Intermittent	n.a.	n.a.	1.6–4	1.3[c]	n.a.	n.a.	50–60[c]
	Continuous	n.a.	n.a.	1.6–4	1.3[c] L/h	n.a.	n.a.	50–60[c]
	Intermittent	n.a.	n.a.	2.42	2.0–2.5[c]	n.a.	n.a.	50–55[c]
	Intermittent	n.a.	n.a.	2.42	2.0–2.5[c]	n.a.	n.a.	50–55[c]
	Continuous	n.a	n.a.	2.52	2.0–2.5[c]	n.a.	n.a.	50–55[c]
[24]								
14 L (9 L active volume)	Continuous	240–40[c]	n.a.	0.5–2.5[c]	n.a.	n.a.	0.327	n.a.
	Intermittent	225–40[c]	n.a	0.5–2.1[c]	n.a.	n.a.	0.314	n.a.
			g VS/L					
Lindmark et al. [51] 1 L (0.7 L active volume)	Continuous	–	13	–	0.52	n.a.	n.a.	–
	Continuous	–	13	–	0.56	n.a.	n.a.	–
	Intermittent	–	13	–	0.54	n.a.	n.a.	–
1 L (0.7 L active volume)	Continuous	–	Inoculum only	–	0.11	n.a.	n.a.	–
	Continuous	–	Inoculum only	–	0.13	n.a.	n.a	–
	Intermittent	–	Inoculum only	–	0.13	n.a.	n.a.	–

n.a Not available.

[a] Calculated from presented data.
[b] Clogging caused a longer retention time of substrate.
[c] Approximated from graph.
[d] Data collected through personal communication.

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